IN VITRO ANTICATARACT ACTIVITY OF *TAMARINDUS INDICA* LINN. AGAINEST GLUCOSE-INDUCED CATARACTOGENISIS

Dissertation submitted to

The Tamil Nadu Dr. M. G. R. Medical University,

Chennai

in partial fulfillment of the award of degree of

MASTER OF PHARMACY

(PHARMACOLOGY)

Submitted by

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Under the guidance of

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MARCH – 2009

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

COIMBATORE – 641 044.

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CERTIFICATE

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This is to certify that the dissertation entitled ***"IN VITRO* ANTICATARACT ACTIVITY OF *TAMARINDUS INDICA* L. AGAINEST GLUCOSE-INDUCED CATARACTOGENISIS."** was carried out by **Mr. SRIKANTH MERUGU,** in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to **The Tamil Nadu Dr. M. G. R. Medical University, Chennai**, under supervision and direct guidance of **Mrs. M. UMA MAHESWARI, M.Pharm., (Ph.D.,)** Department of Pharmacology, College of Pharmacy, SRIPMS, Coimbatore – 44.

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Srikanth Merugu

LIST OF ABBREVIATIONS

ANOVA	:	Analysis of variance
ARI	:	Aldose reductase inhibitors
CAT	:	Catalase
g	:	Gram
GPx	:	Glutathione peroxidase
GSH	:	Reduced glutathione
GSSH	:	Glutathione reductase
HCl	:	Hydrochloric acid
KCl	:	Potassium chloride
LH	:	Lipid hydroperoxides
LPO	:	Lipid peroxidation
MDA	:	Malondialdehyde
mg/dl	:	milligram/deciliter
µg	:	Microgram
µM	:	Micromolar
NSAIDs	:	Non-steroidal anti-inflammatory drugs
ROS	:	Reactive oxygen species

SOD	:	Superoxide dismutase
TBA	:	Thiobarbituric acid
TBARS	:	Thiobarbituric acid reactive substances
T. INDICA L	:	*Tamarindus indica* Linn.
UV	:	Ultra violet

INTRODUCTION

The crystalline lens is a biconvex, vascular, transparent structure enclosed by a capsule, a basement membrane secreted by the lens epithelium. The capsule, responsible for moulding the lens substance during accommodation, is thickest in the equatorial zone and thinnest at the posterior pole of the lens. The normal lens is transparent and any congenital or acquired opacity in the lens capsule or substance, irrespective of the effect on vision, is a cataract (Kanski, 2003).

Cataract is the opacification of lens often associated with old age and is a major complication of diabetes mellitus because higher glycosylated hemoglobin levels are significantly associated with increased risk of cataract (Langade, 2006).

TYPES OF CATARACT

A. Acquired cataract

1. Age releated cataract

a.) Morphological classification

i) Subcapsular cataract

Anterior subcapsular cataract lies directly under the lens capsule and associated with fibrous metaplasia of the lens epithelium.

Posterior subcapsular cataract lies just infront of the posterior capsule and manifests a vacuolated, granular or plaque-like appearance. Patients are particularly troubled under conditions of miosis, such as produced by headlights of oncoming cars and bright sunlight. Near vision is also frequently impaired more than distant vision.

ii) Nuclear cataract starts as an exaggeration of the normal ageing changes involving the lens nucleus. It is often associated with myopia due to an increase in the refractive index and also with increased spherical

aberration. Some elderly patients may consequently be able to read again without spectacles.

iii) Cortical cataract may involve the anterior, posterior or equatorial cortex. The opacities start as clefts and vacuoles between lens fibres due to hydration of the cortex. Both cortical and subcapsular cataracts are white on oblique illumination and appear black, silhouetted against the red reflex, on retroillumination.

b) Classification according to maturity

i) An immature cataract is one in which the lens is partially opaque.

ii) A mature cataract is one in which the lens is completely opaque.

iii) A hypermature cataract has a shrunken and wrinkled anterior capsule due to leakage of water out of the lens.

iv) A morgagnian cataract is a hypermature cataract in which total liquefaction of the cortex has allowed the nucleus to sink inferiorly (Hejtmancik, 2004).

2. Presenile cataract

Cataract may develop early in the following conditions,

a.) Diabetes mellitus

Classical diabetic cataract is rare. Hyperglycemia is reflected in a high level of glucose in the aqueous humor, which diffuses into the lens. Here glucose is metabolized by aldose reductase into sorbitol, which then accumulates within the lens, resulting in secondary osmotic over hydration of the lens substance. Nuclear opacities are common and tend to progress rapidly.

Premature dystrophy may be seen due to reduced pliability of the lens.

b.) Myotonic dystrophy

About 90% of patients develop visually innocuous, fine cortical iridescent opacities in the third decade, which evolve into visually disabiling stellate posterior subcapsular cataract by the fifth decade. Occasionally cataract may antedate myotonia.

c.) Atopic dertmatitis

About 10% of patients with severe atopic dermatitis develop cataracts in the second to fourth decades. The opacities are often bilateral and may mature quickly. Shield – like anterior subcapsular plaque which wrinkles the anterior capsule is characteristic. Posterior subcapsular opacities resembling a complicated cataract may also occur. Neuro fibromatosis type 2 is associated with posterior subcapsular or posterior cortical opacities.

3. Traumatic cataract

Trauma is the most common cause of unilateral cataract in young individuals. The following may be responsible,

a) Direct penetrating injury to the lens.

b) Concussion may cause an imprinting of iris pigment on the anterior lens capsule (Vossius ring) as striking flower – shaped cortical opacities (rosette cataract)

c) Electric shock and lightining are rate causes.

d) Ionizing radiation to ocular tumours.

e) Infrared radiation- If intense as in glassblowers may rarely cause true exfoliation or lamellar delamination of the anterior lens capsule in which the superficial portion of a thickened capsule splits from the deeper layer and extends into the anterior chamber.

B. Drug – induced cataract

a) Steroids, both systemic and topical, are cataractogenic, the lens opacities are initially posterior subcapsular, later the anterior subcapsular region becomes affected. The relationship between weekly systemic dose, duration of administration, total dose and cataract formation is uniclear. It is through that patients on less than 10 mg prednisolone (or equivalent) or treated for less than 10 mg prednisolone (or equivalent), or treated for less than 4 years may be immune. Although it is believed that children may be more susceptible to the cataractogenic effects of systemic steroids, individual genetic susceptibility may also be of relevance. It has therefore been suggested that the concept of a safe dose be abandoned. Patients who develop lens changes should have their dosage reduced to a minimum, consistent with control of the underlying disease, and if possible be considered for alternate drug therapy. Early

opacities may regress if therapy is discontinued, alternatively progression may occur despite withdrawn and warrant surgical intervention.

b) Chlorpromazine may cause the deposit of innocuous fine, stellate, yellowish – brown granules on the anterior lens capsule within the papillary area. Diffuse, granular deposits on the corneal endothelium and in the deep stroma may also occur. Both lenticular and corneal deposits are dose –related and usually irreversible. In very high doses (>2400 mg daily), this drug may cause retinotoxicity.

c) Busulphan (Myleran) used in the treatment of chronic myeloid leukaemia, may occasionally cause lens opacities.

d) Amiodarone, used in the treatment of cardiac arrhythmias, causes visually inconsequential anterior subcapsular lens deposits in about 50% of patients on

moderate to high doses. Vortex keratopathy may also occur.

e) Gold, used in the treatment of rheumatoid arthritis, causes innocuous anterior capsular deposits in about 50% of patients on treatment for longer than 3 years.

f) Allopurinol, used in the treatment of hyperuricaemia and chronic gout, increases the risk of cataract formation in elderly patients, if the cumulative does exceeds 400 g or duration of administration exceeds 3 years.

C. Secondary cataract

A secondary (complicated) cataract develops as a result of some other primary ocular diseases.

i. Chronic anterior uveitis is the most common cause of secondary cataract. The earliest finding is a polychromatic luster at the posterior pole of the lens which may not progress if the uveitis is arrested. If the

inflammation persists, posterior and anterior opacities developed may progress to maturity. Lens opacities appear to progress more rapidly in the presence of posterior synechiae.

ii. Acute congestive angle - closure glaucoma may cause small grey whiteanterior, subcapsular or capsular opacities within the papillary area (glaukomflexken). They represent focal infarcts of the lens epithelium and are pathognomonic of past acute angle closure glaucoma.

a. High (Pathological) myopia is associated with posterior subcapsular lens opacities and early–onset nuclear sclerosis, which may ironically increase the myopic refractive error. Simple myopia, however, is not associated with such cataract formation.

b. Hereditary fundus dystrophies such as retinitis pigmentosa, Leber congenital amaurosis, gyrate atrophy and stickler syndrome may be associated with posterior subcapsular lens opacities. Cataract surgery may

occasionally improve visual acuity even in the presence of severe retinal changes (Kanski, 2003).

Epidemiology of cataract in India

Cataract remains the leading cause of visual disability and blindness all over the globe (Gupta *et al.*, 1997a). The problem is more acute in the developing countries. It is estimated that there are about 12 million blind people in India alone due to cataract. Even though surgical removal of cataractous lens with the use of corrective lenses has helped and provide means to lessen the problem of vision loss, these procedures are expensive and not affordable for many in developing countries. Moreover, lack of resources such as medical expertise, equipment, supplies, etc. has limited the availability of corrective measures for cataract related blindness in many countries.

Cataract, a multi factorial disease occurs mainly due to the formation of large protein aggregates in the lens. It is due to the post translational modifications of lens crystalline such as oxidation, glycation, Schiff's base formation, carbamylation, transamidation, phosphorylation and proteolysis leading to clouding of the lens. Hydration has been shown to be a common factor in development of sugar, microwave ionizing radiation, naphthalene mouse cataracts, triparanol and hereditary (Junakar, 1998).

Risk factors

Risk factors are many for cataract development in humans. Aging is a prevalent risk factor in the development of cataracts. High incidences of cataracts have been observed in aged individuals. Apart from aging, genetic factors, nutrition, diarrhea, diabetes, trace metals, ultra violet radiation, glaucoma, hypertension,

myopia, alcohol, and smoking have been implicated as significant risk factors in the causation of cataract (Gupta *et al.*, 1997a; Junakar, 1998).

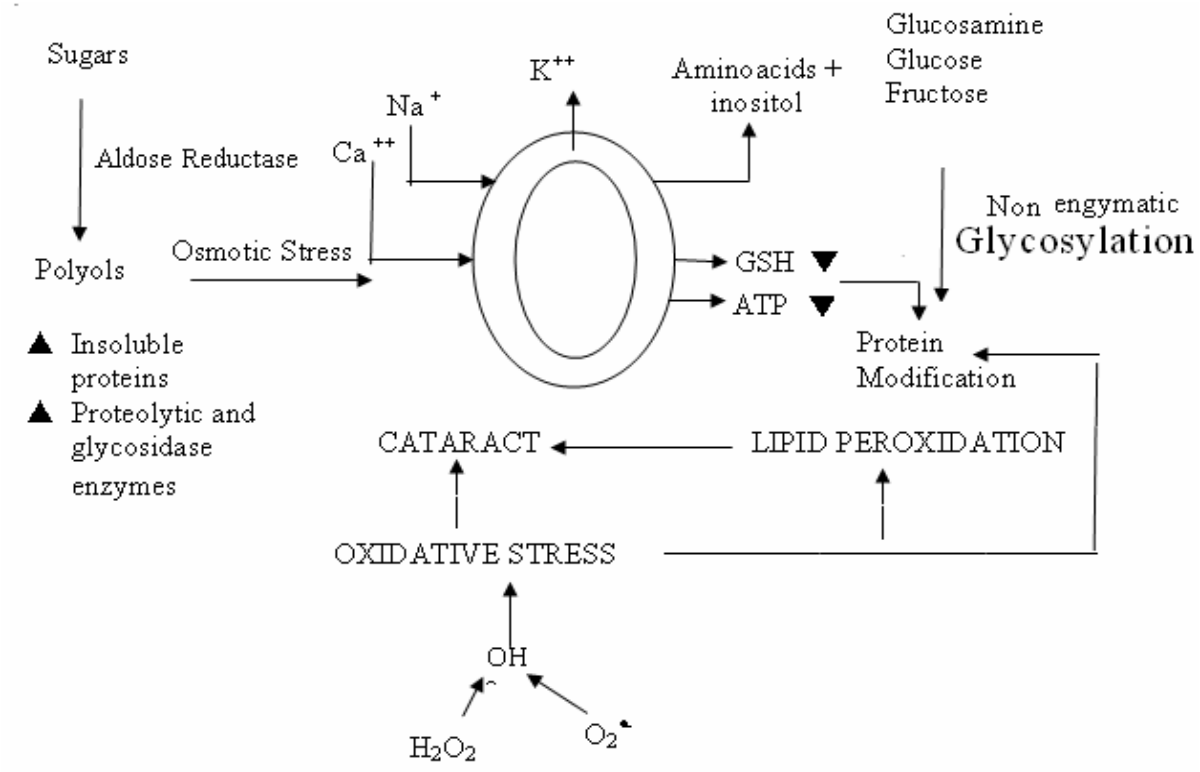
Mechanisms associated with cataract

There are mainly two mechanisms involved in the development of cataract. Trypotophan which is an amino acid is present in eye lens in higher concentration. When it absorbs UV radiation, it forms N- Formyl Kynurenine. It may combine with the 3-OH Kynurenine and riboflavin which are lens photosensitizers, they absorb photons form the light and emits electrons. These electrons react with the molecular O_2 to form super oxide anion radical which reacts with Na^+ K^+ ATPase pump in eye and may cause the swelling of the eye and cause lens opacification.

The other mechanism may be due to the oxidation of cysteine which is an aminoacid, resulting in the formation of the disulphide bond between the crystalline proteins

which may produce insoluble aggregates which may cause lens opacification (Rees and Pirie, 1967; Gupta *et al.*, 1997a; Halliwell, 2001).

Mechanisms Involved in Cataractogenesis(Gupta *et al.*, 1997a)



FREE RADICALS INVOLVED IN CATARACTOGENESIS

Free radicals may be formed either by the reduction of molecules by electron transfer or by the hemolytic

cleavages of covalent bond. Both these reactions may be enzymatic or non –enzymatic.

Due to the presence of an odd unpaired electron in its outermost orbital, these free radicals are unstable and readily react with neighborhood molecules and extract electrons from them, converting the attacked molecule into a few radical, which inturn attacks another molecule generating more free radicals and so on. This enables free radicals to induce chain reactions that may be thousands of events long.

A free radical reaction is terminated by reaction between two free radicals or neutrilization by antioxidants (Uday *et al.*, 1999).

Generation of Free radicals

Biological free radicals include reactive oxygen species, reactive nitrogen species, reactive sulfur species, free radicals obtained form xenobiotics.

a.) Superoxide anion radical ($O_2^{\cdot -}$)

It is generated from NADPH oxidase and from mitochondria.

i) NADPH oxidase is present in the lysosomal cell membrance. It steals electron from O_2 results in the formation superoxide anion radical (O_2). It is converted to hydrogen peroxide and is a spontaneous reaction which known as respiratory burst. This hydrogen peroxide may react with the chlorine in the presence of myeloperoxidase to form hypochlorous acid or it may produce hydroxyl radicals, by the Fenton reaction which uses the metal ion Fe^{3+}. They are capable of killing bacteria.

ii) From Mitochondria: Ubiquionone, which is a terminal acceptor of electron, is converted to semiquinone (free radical). By reacting with O_2, it forms (O_2·-) super oxide radical and with H_2O_2, it forms hydroxyl radical.

b.) Hydrogen Peroxide

It is formed by the dismutation of superoxide by the enzyme superoxide dismutase.

$$O^{\cdot}{}_2^- + O^{\cdot}{}_2^- \xrightarrow{SOD} H_2O_2$$

Hydrogen peroxide is generated from

i) Aminoacid oxidases: Flavin is a co-enzyme required for the oxidative deamination of aminoacid. The reduced flavin attacks molecular oxygen to form hydrogen peroxide.

ii) Xanthine oxidase: Xanthine oxidase catalyses the convertion of hypoxanthine to xanthine in the presence of

xanthine oxidase and hydrogen peroxide which comes from molecular oxygen.

iii) Peroxisomes: Peroxisomes is the site of β-oxidation of fatty acids. β- Oxidation of the fatty acids is catalysed by acetyl co-enzyme-A dehydrogenase. During this process, a co-enzyme called FAD which donates two electrons gets reduced to $FADH_2$. Again it is converted to FAD. During that process it gives out O_2 and H_2O (Kovaceva *et al.,* 2007).

c.) Hydroperoxyl radical

They are highly lipophillic and capable of initating lipid peroxidation.

LIPID PEROXIDATION

Lipid peroxidation is a self-perpetuating widespread process through which lipid components of the membranes from cell organelles are converted into lipid

peroxides an event which strikingly contribute towards the formation of lipofuscin pigment (Nordmann, 1994). The membrane debris derived from cell membrane system including organelles, while lysosomic ROS are the end-result of a complex oxidative chain of molecular event mainly initiated in mitochondria during energy production. More specifically, H_2O_2 generated by mitochondria in smaller amounts by other organelles, pervade the lumen of lysosomes. Fenton-type reaction between H_2O_2 and Fe (II) take place leading to the production of the harmful hydroxyl radicals, which are capable of initiating processes of lipid peroxidation (Halliwell, 2001). Malondialdehyde is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids (PUFA). MDA, a secondary product of LPO, is used as an indicator of tissue damage by a series of chain reactions. MDA is also a by-product of prostaglandin biosynthesis. It reacts with thiobarbituric acid and produces a red-coloured product. MDA is a

mutagenic and genotoxic agent that may contribute to the development of human cancer.

IN VIVO MODELS IN CATARACT (Gupta, 2004)

1. Sugar catract

i) Galactose – induced cataract

The changes associated with galactose cataractogenesis include the initial reduction of galactose into dulcitol through intervention of aldose reductase with NADPH as a co-factor. Accumulation of dulcitol in the lens, (since it is not metabolized) creates cellular hypertonicity associated with and/or followed by a cascade of events, which includes an influx of water, swelling of the lens fibres, epithelial cell edema, damage of plasma membrance, compromise of cellular permeability, a drop in myinositol level, a reduction in Na^+ K^+ ATPase activity an influx of Na^+ and Cl^- and an efflux of K^+ and the loss of glutathione and aminoacids. These are

the morphological, biochemical, enzymatic and molecular alterations in the lens associated with galactose cataracts.

ii) Alloxan – induced cataract

Alloxan is a cyclic urea analog which is highly reactive molecule that is readily reduced to dialuric acid, which is then auto oxidized back to alloxan resulting in the production of H_2O_2, O_2^{-} and hydroxyl radical. However, the other mechanism reveals the ability of alloxan to react with protein sulfhydryl groups on hexokinase, a singnal recognition enzyme in the pancreatic β cell that couples changes in the blood glucose concentration to the rate of insulin secretion. By this mechanism, inhibition of glucokinase and other SH containing membrane proteins on the β- cell would eventually result in cell necrosis within minutes.

iii) Streptozocin – induced cataract

Diabetes reated cataractogenic changes are seen in the animals injected with streptozocin. The chemical structure of streptozocin has a glucose molecule with a highly reactive nitrosourea side chain, which supposedly initiates its cytotoxic action. The glucose moiety directs this agent to the pancreatic β cells. There it binds to the membrance recptor to generate structural damage. At the intracellular level three major phenomena are responsible for β cell death,

i) Process of methylation

ii) Free radical generation

iii) Nitric oxide (NO) production.

The damage caused to β cells alters the sugar metabolism leading to diabetes.

2. Selenite – induced cataract

Selenite cataract resembles human cataract in many ways such as vesicle formation, increased calcium, insoluble protein, decreased water–soluble proteins and reduced glutathione (GSH). However, selenite cataract shows no high molecular weight protein aggregation or increased disulfide formation and is dominated by rapid calpain-induced proteolytic precipitation, while semile cataracts may be produced by prolonged oxidative stress.

3. Naphthalene – induced cataract

Naphthalene is oxidized in the liver first to an epoxide and then it converted into naphthalene dihydrodiol. This stable component is converted enzymatically into dihydroxynaphthalene to reaching the eye. Being unstable at physiological pH, 1, 2-dihydroxynaphthalene and spontaneously autooxidises to

1, 2- naphthoquinone and H_2O_2 . It alkylates proteins, glutathione and aminoacids and generates free radicals.

4. Glucocorticoid – induced cataract

Glucocorticoid cataract results in the formation of steroid– adduct protein, induction of transglutaminase and reduction of ATPase activity may lead to cataract. Steroid cataracts are produced by the activities of glucocorthicoids and progressed by way of production of oxidative stress similar to other types of cataract.

5. L- Buthionine – S, R- Sulfoximine (BSO) - induced cataract

Glutothione is present in mammalian lens in high concentrations and is involved in the protection of lens against oxidation. In most of the cataracts the decrease in its level is observed.

6. Smoke – induced cataract

Cigarette smoke contains trace and heavy metals. The increased metal contents in lens cause lens damage by the mechanism of oxidative stress -forming oxygen radicals, via metal catalyzed fenton Reaction. In other words cigarette smoke is associated with the accumulation of iron and calcium.

7. UV radiation – induced cataract

Epidemiological studies have shown a link between exposure to UV radiation in sunlight and development of cataract. Experimental studies confirm that ultraviolet (UV) radiation induces cataract. There is, however, a lack of data on the age dependence in experimental UV radiation-induced cataract.

8. Microwave – induced cataract

Microwave radiation has been reported to produce posterior subcapsular and cortical cataracts in rabbits and dogs within a short span of time.

9. Transforming Gowth Factor β (TNFB) – induced cataract

TGFB is induced by injecting approximately 60 ng TGFB into the vitreous. TGFB stimulates lens epithelial cells to undergo aberrant morphologic and molecular changes that mimic the changes observed in human posterior subcapsular and cotical cataract (Gupta, 2004).

***IN VITRO* MODELS IN CATARACT (Gupta, 2004)**

Induction of cataract in isolated animal lenses maintained in organ culture has become a convenient, quick and appropriate method for testing the anticataract efficacy of an agent. Opacification of lens is

induced by generating oxidative stress/ hyperglycemic/ hypergalactosemic conditions around the lens by supplementing the culture medium with a variety of exogenous substances.

1. Oxidative stress – induced cataract

Oxidative mechanisms play an important role in many biological phenomena including cataract formation. Formation of the superoxide radical in the aqueous humor, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to the formation of cataract. *In vitro* such cataracts are induced by agents like selenium, H_2O_2, photosensitizers and enzyme xanthine oxidase.

2. Selenite - induced cataract

In vitro cataract is produced by supplementing the tissue culture medium with 25 to 100 mM sodium selenite in

which freshly enucleated transparent rat lenses are incubated at 37^0C. This causes membrane damage and faint cortical opacities within 24 h.

3. Photochemically - induced cataract

Riboflavin, a photosensitizer, is supplemented in the culture medium to induce cataract in cultured lenses. Micro quantities (4-200 μM) of riboflavin lead to servere physiological damage and opacification within 24 h after exposure to light. The initial membrane damage is evidenced by a disturbed cation ratio between lens water and the medium of incubation. Riboflavin on getting photosensitized generates free radicals in a sequence of reactions.

Lenses are maintained in organ culture for 24 to 72 h. The lenses are divided into four groups and incubated in the dark and light both in presence and absence of riboflavin. The lenses are exposed to light with two 15-w

daylight fluorescent lamp placed at 8 inches above the cluster plate. The culture medium is replaced every 24 h. Riboflavin shows no effect on the lens in the absence of light, and light without riboflavin has no significant effect. opacification starts in the equatorial zone and gradually extends towards the centre of the lens.

4. Enzymatically - induced cataract

Supplementation of culture medium with 1 mM xanthine and 0.1 unit xanthine oxidase, which act as substrate and enzyme respectively, leads to generation of superoxide radical. The lenses suffer severe oxidative damage and turn opaque within 24 h when incubated in culture medium at 37^0C.

5. Hydrogen peroxide – induced cataract

Incubation of lenses in medium containing 50-500 μM H_2O_2 produces cataract. opacification starts in the

equatorial region within 24 h. The entire superficial cortex becomes opaque by 96 h. Due to the high instability of H_2O_2, the medium is changed every 2 h during the first eight hours.

6. Sugar – induced cataract

Transparent and undamaged lenses are incubated in a basis culture medium with fetal calf serum for 24 to 48 h. In the control group the medium is supplemented with glucose (30 mM), lenses develop opacity in the subcapsular region on day 1 and in the central region on day 2. Biochemical analyses reveal raised polyol, malondialdehyde levels and water content, and decreased glutathione levels in these lenses.

7. Steroid – induced cataract

Steroid–induced experimental cataract is produced *in vitro* by incubating the transparent lenses in the medium

containing methyl prednisolone (1.5 mg/ml). The test agent and methyl prednisolone added alone and together to the medium form drug control, control and treated groups respectively. Early cataract around the equator is produced within 24 h of incubation. Incubation period may be extended to 48 h for dense opacity. Morphological changes and modulation in biochemical parameters between the groups may show the potential of the anticataract agent.

8. Naphthalene – induced cataract

TC–199 medium is used for the preincubation of lens. Stock solution of napthalene dihydrodiol is prepared in 20% ethanol at 2.5×10^{-3} M concentration. The stock solution is diluted 1:100 to obtain the final concentration of 25.5×10^{-5} M. The final osmolarity of the solution is 295-300 m Osmol. Rat lenses are incubated in TC-199 medium containing napthalene metabolite solution. Medium is

renewed daily till 72 h. Lenses remain clear during the initial 24 h but from shell–like opacity around the nucleus by 48 h. Opacification becomes more peripheral and widespread after 72 h. At 48 h, under such conditions of incubation, development of opacity mimics the *in vivo* napthalene cataract. Naphthalene is oxidized in the liver first to an epoxide and then is converted into naphthalene dihydrodiol. This stable component on reaching the eye gets converted enzymatically to dihydroxynaphthalene. Being unstable at physiological pH, 1,2 dihydroxynapthalene sponaneously auto oxidises to 1,2 naphthoquinone and H_2O_2. It alkylates proteins glutathione and amino acids and generates free radicals. There is a loss of protein thiol in this reaction and the products are less easily digestible by pancreatin than normal lens protein (Rees and Pirie, 1967).

9. Ca^{++} - induced cataract

In this model, the control group contains the lenses incubated in the medium enriched with 20 mM Ca^{2+} or 1x 10^{-2} mM A23187 calcium ionopore. The treatment group lenses are cultured in the calcium and the test drug-containing medium. Incubation period can range from 24-72 h (Gupta, 2004).

PHARMACOLOGICAL STRATEGIES FOR PREVENTION OF CATARACT

Drugs have been developed which are aimed to interact at the level of altered lens metabolism and lens pathophysiology. The anti cataract agents claimed to be effective *in vitro*, *in vivo* and in epidemiological studies may be broadly classified in the following categories (Gupta *et al.*, 1997b).

- Aldose reductase inhibitors
- Nonsteroidal anti –inflammatory drugs

- Vitamins, minerals and antioxidants
- Agents acting on glutathione
- Miscellaneous agents.

1) Aldose Reductase Inhibitors

These drugs are aimed to block the metabolic pathways of glucose responsible for diabetic vascular dysfunctions. Aldose reductase inhibitors prevents the accumulation of sorbital within the lens would have an osmotic effect bringing in water and causing swelling and opacification. Sorbinil a spirohydantoin became the most powerful sorbitol lowering agent. Sorbinil prevents increased fluorescence and protein aggregation and it also acts as an antioxidant.

2) Non Steroidal Anti –Inflammatory Drugs

The NSAIDS extensively studied are aspirin, paracetamol, Ibuprofen, naproxen, sulindac and

bendazec. The mechanism associated with the protective effect of NSAIDS includes acetylation, inhibition of glycosylation and carbamylation of lens protein. Some of them are also reported to inhibit lens AR to varying extent. NSAIDS have also been reported to possess antioxidant properties. Most of the studies on the evaluation of anticataract potential of drugs have been conducted by feeding the drugs by oral route.

3) Agents which act on glutathione

The concentration glutathione a tripeptide thiol decreases with age in the lens and more markedly in cataract. Glutathione has been reported to control calcium influx and protect lens protein against damaging effects of sugars. phaken is a preparation containing there constituents of aminoacids glutathione plus agrinine, inositol, pyridoxine and ascorbic acid.

4) Vitamins, minerals and antioxidants

If oxidation in lens leads to cataract formation, then is feasible to prevent it by the use of antioxidants such as vitamins E and C and perhaps β-carotene. The potential role of vitamins and antioxidants in preventing various diseases is well documented there are reports suggesting beneficial effect of vitamins like C and E in preventing cataract. Beta –carotene has also been demonstrated to protect lens damage by hematoporphysin. Ascorbate protects rubidium uptake against free radical damage and prevents light induced protein cross linking. Protective effect of vitamin C has been also reported in various *in vitro* studies. vitamin E has been found to delay cataractogenesis in diabetic rats and in Emory mouse. Vitamins C and E, β- Carotene and other anticataract agents probably act via a common mechanism of their scavenging properties of free radicals (Gupta *et al.*, 1997b).

ANTIOXIDANT ENZYMES

1) Superoxide Dismutase (SOD)

SODs are a family of metalloenzymes that converts superoxide to hydrogen peroxide (H_2O_2) and represents the first line of defense against oxygen toxicity.

$$2O_2^- + 2H \rightarrow H_2O_2 + O_2$$

Three forms of SOD have been described. The first isoform, containing copper and zinc at its active site (Cu/Zn SOD-1), is found in the cytoplasm of cells. Another isoform, containing manganese at its active site is located in mitochondria (Mn SOD-2). The third isoform is present in extracellular fluids such as plasma (Cu/Zn SOD-3). SOD is a stress protein, which is synthesized in response to oxidative stress. It was found that the traces of copper, zinc and manganese metals are essential for maintaining the antioxidant activity of SOD (Halliwell, 1994; Ray and Husain, 2002).

2) Glutathione Peroxidase (GPx)

GPx is one of the major enzymes responsible for the degradation of hydrogen peroxide and organic peroxides in the brain. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. There are two isoforms have been identified, selenium-dependent which is highly active towards H_2O_2 and organic hydroperoxides and selenium independent GPx. GPx activity has been reduced in selenium deficiency (Muller *et al.,* 1984; Son *et al.*, 2007).

3) Catalase (CAT)

It is a heme-containing protein present in most cells.

$$2H_2O_2 + 2H_2O \rightarrow O_2$$

Catalase is 104 times faster than GPx. It consists of four protein subunits, each containing a heme Fe (III)-protoporphyrin group bound to its active site. GPx and

CAT were found to be important in the inactivation of many environmental mutagens (Ray and Husain, 2002).

4) Glutathione (GSH)

GSH is a ubiquitous tri-peptide formed from three aminoacids glutamate, glycine and cysteine and synthesized by two ATP-dependent enzymatic reactions. GSH has major intracellular antioxidant molecule. It plays a critical role in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH transferase. It was shown that depletion of GSH enhances cerebral ischemic injury in rats (Mizui ***et al.***, 1992; Son ***et al.***, 2007).

PLANT PROFILE

***Tamarindus indica* Linn.**

Family : Fabaceae

Vernacular names

Hindi : Amli, Amlica

Sanskrit : Abdika, Amla

Tamil : Ambilam, Puli

Telugu : Amlika, Chintha chettu

Marathi : Ambali, Chicha

Biological source:

Dried leaves of *Tamarindus indica* Linn.

Distribution:

The tree has long been naturalized in the East Indies and the islands of the Pacific. Tamarind is indigenous to

tropical Africa, Bermuda and the Bahamas. It is cultivated throughout India and West Indies (Morton *et al.,* 1987).

Fig. 1 *Tamarindus indica* Linn. leaves and fruits

Fig. 2 *Tamarindus indica* Linn. flowers

Description:

It is a large tree 12-18m high; branches spreading, glabrous. Leaves are 5-12 cm long; rachis slender, channeled; stipules linear, caduceus. Leaflets subsessile, 10-20 pairs, tolerably loosely set on veined. Flowers in lax few-flowered racemes at the ends of the branchlets;

pedicels 6-10 mm long, enclosing the buds. Calyx 1.3 cm long. Petals 3 (an upper and 2 lateral) 1 cm long, subequal, obovate-oblong. Ovary stalked;ovules 8-12 or more. Seeds 3-12, obovate-oblong, truncate at the ends (Krithikar and Basu, 1981; Morton *et al.*, 1987).

Parts used:

Leaves, seeds, fruits, bark, flowers.

Chemical constituents:

Pulp contains free organic acid (about 10% of tartaric citric and malic), a little nicotinic acid and about 30-40% of invert sugar. Flavonoid C-glycosides (vitexin, isovitexin,orentin and isovetxin) in leaves (Trease and Evans, 2002).

Uses:

The leaves are applied to reduce inflammatory swellings, ringworm, useful in diseases of the blood, smallpox, ear ache ophthalmia and other eye diseases. Fresh leaves crushed with water and expresses yield fluid which is said to be useful in bilious fever and scalding of the urine.

The bark is used topically for loss of sensation in paralysis; the ash is given for urinary discharges and gonorrhoea. The bark has astringent and tonic properties, heals ulcers.

The fruit is sour, sweetish and bitterish, laxative, useful in liver complaints, vomiting, scabies, sore throat, stomatitis. The pulp of the fruit is tonic to the heart, astringent and aperient, useful for checking bilious vomiting. The ripe fruit is regarded as refrigerant, digestive,

carminative and laxative, and useful in diseases supposed to be caused by deranged bile, such as burning of the body, costiveness, intoxication from spirituous liquors or dhatura, etc. The unripe fruit is sour, tasty, and astringent to the bowels.

The flowers are acrid, sweet, sour, tasty, appetizing, cure “vata” and “kapha”, urinary discharges, bad odour in perspiration. The seeds are said to be a good astringent, boiled seeds are used as a poultice to boils; pounded seeds are applied to the crown of the head in cough and relaxation of the uvula (Krithikar and Basu, 1981).

REVIEW OF LITERATURE

Umamaheswari and Chattejee (2008) investigated the effect of the fractions of *Coccinia grandis* on naphthalene-induced cataractogenesis in rats. The cataract progression due to naphthalene feeding was monitored using an ophthalmoscope and classified in to 5 stages. At the end of the experiment, levels of malondialdehyde, lipid hydroperoxides, and carbonyl and sulfhydryl content, enzymatic and non-enzymatic antioxidants in lens homogenate were measured. In addition, there was a significant increase in lipid peroxidation and protein carbonyl content and a decrease in protein sulfhydryl content and antioxidant enzymes when compared with healthy controls. The results indicates, the animals treated with naphthalene showed a varying degree of ctaractogenic changes as evidenced by about 66.6% of animals in stage 4 and

33.3% in stage 5 on 5 on the 28th day of treatment. none of animals treated with the petroleum ether, chloroform and ethyl acetate fractions of *C.grandis* showed mature stage 5 cataracts on the 28th day.

Raju *et al.*, (2007) investigated the influence of kynurenines in pathogenesis of cataract formation in tryptophan–deficient regimen in *Wistar* rats. L- Tryptophan is an essential amino acid and its deficiency is involved in various pathologies. The rats were maintained on tryptophan deficient diet and there was decrease in protein content, kynurenines, reduced glutathione (GSH), glutathione peroxidase (GPx), glutatione-S-tranferase (GSTs) and tryptophan-fluoresence intensities and an increase in lipid peroxidation indicative of oxidative stress. The above changes were normalized in the rats on supplementation of 0.05% tryptophan in their diets. These results suggest that tryptophan-deficiency in the diet leads to an overall significant decrease in kynurenines and

levels of antioxidant enzymes (except SOD) in ocular tissue with a concomitant lenticular opacification. The results suggest that diet with adequate tryptophan has protective influence and is of immense benefit in mitigating the changes that may otherwise contribute to lenticular opacities.

Son *et al.,* (2007) reported that taurine prevents oxidative damage of high glucose-induced cataractogenesis in isolated rat lenses. taurine has antioxidant capacity and its level in diabetic cataractous lens in markedly decreased. Taurine is a part of antioxidative defense mechanism involved in protecting the lens against high glucose-induced oxidative stress and tissue damage. Lenses were isolated from male *Sprague-Dawley* rats weighing 180-200g and cultured in high-glucose medium. The culture of lenses in high glucose medium increased the weight and opacity of lenses and the carbonyllated protein level, and decreased

glutathione (GSH) content. Although there were no significant effects of taurine on the weight or opacity of lenses, pretreatment of lenses with taurine significantly reversed the level of protein carbonyl and GSH to those of controls.

Doughari (2006) evaluated the antimicrobial activity of *Tamarindus indica* Linn. The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its antimicrobial activity. The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria and fungi using the paper disc diffusion method. The results of the phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and phlobatamins and the extracts were active against both gram positive and gram negative bacteria. The activity of the plant extracts were not affected when

treated at different temperature ranges (4°C, 30°C, 60°C and 100°C), but was reduced at alkaline pH. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC and the MBC were demonstrated against *Salmonella paratyphi, Bacillus subtilis* and *Salmonella typhi* and the highest MIC and MBC was exhibited against *Staphylococcus aureus*. *Tamarindus indica* has broad spectrum antibacterial activity and potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

Gritz *et al.*, (2006) investigated the effect of antioxidant supplement in the prevention of cataract. The primary outcome variable was change in nuclear opalescence over time. Secondary outcome variables were cortical and posterior subcapsular opacities and nuclear colour changes; best corrected visual acuity

change; myopic shift; and failure of treatment. Annual examinations were performed for each subject by three examiners, in a masked fashion. Multivariate modelling using a general estimating equation was used for analysis of results, correcting for multiple measurements over time. The results Initial enrolment was 798 subjects. Treatment groups were comparable at baseline. There was high compliance with follow up and study medications. There was progression in cataracts. There was no significant difference between placebo and active treatment groups for either the primary or secondary outcome variables. Antioxidant supplementation with b carotene, vitamins C and E did not affect cataract progression in a population with a high prevalence of cataract whose diet is generally deficient in antioxidants.

Langade *et al.*, (2006) studied the *in vitro* prevention using ACE inhibitors of cataract induced by glucose. Goat lenses were incubated in artifical aqueous humor

containing 55 mM glucose with lisinopril or enalapril in different concenterations at room temperature for 72 h. Biochemical parameters studied in the lens were electrolytes (Na^+, K^+) Na^+ K^+-ATPare activity, Malondialdehyde (MDA) and proteins. Glucose-induced opacification of goat lens began 8-10 h after incubation and was complete in 72-80 h. Cataractous lenses showed higher Na^+, MDA ($p<0.001$), lower Na^+ - K^+ -ATPase activity and water soluble protein content. Lenses treated with lisnopril or enalapril in concentrations of 1, 5 and 10 ng/ml showed higher protein (total and water soluble proteins) content and prevented formation and progress of cataract by glucose as evidenced by biochemical parameters. The anticataract activity of lisnopril and enalapril may be because of the antioxidant and free radical scavenging activity, as evidenced by a decrease in MDA in treated lenses. Further *in vitro* and *in vivo* studies in various experimental models and long term clinical trials

are required to validate the anti cataract activity of ACE-inhibitors.

Kyselova *et al., (*2005a) investigated the temporal relationship between lens protein oxidation and cataract development in streptozotocin-induced diabetic rats. By the end of the 17th week, approx 50% of the diabetic animals developed mature cataract. In the course of lens opacification, they observed a time-dependent increase in the content of protein carbonyls ($P<0.01$) and decrease in the concentration of protein sulfhydryl ($P<0.001$) content was found in the lenses of diabetic animals. The main finding of their study was the disclosure of quantitative relationship between the degree of protein oxidation and the rate of advanced cataract development in the widely used model of streptozotocin-induced experimental diabetes in rats.

Kyselova *et al.*, (2005b) investigated the effect of dietary supplementation with the pyridoindole antioxidant stobadine on the development of streptozotocin- induced diabetic cataract. The male *Wistar* rats were fed for 18 weeks a standard diet or a diet supplemented with stobadine (0.05% w/w), vitamin E (0.1% w/w), Butylated hydroxy toluene (BHT 0.4% w/w) or a mixture of stobadine (0.05% w/w) and vitamin (0.1% w/w). The progress of cataract was monitored biweekly opthalmoscopic inspection. The content of the free sulfhydryl and carbonyl was determined in total lens proteins. It shows a significant diminution of sulfhydryl groups and elevation of carbonyl groups in diabetic animals in comparison to healthy controls. In diabetic animals, stobadine supplementation significantly attenuated plasma levels of malondialdehyde, an index of systemic oxidative damage. They reported that the ability of stobadine to attenuate lipoxidation reactions in diabetes may

account, at least partly, for its observed anticataract actions.

Komutarin *et al.*, (2004) reported the inhibition of nitric oxide production by murine macrophages from the extract of the seed coat of *Tamarindus indica*. The seed coat extract of *Tamarindus indica*, a polyphenolic compound, has been shown to have antioxidant properties. The studies investigated the inhibitory effect of the seed coat extract of *T.indica* on nitric oxide production *in vitro* using a murine macro-phage like cell line, RAW 264.7, and *in vitro* and *in vivo* using freshly isolated B6C3F1 mouse peritoneal macrophages. *In vitro* exposure of RAW 264.7 cells or periotonial macrophages to 0.2-200 µg/ml of *T.indica* extract significantly attenuated (as much as 68%) nitric oxide production induced by lipopolysaccharide (LPS) and interferon gamma in a concentration dependent manner. The studies suggest that in mice, *T.indica* extract at

concentrations upto 500 mg/kg may modulate nitric oxide production in the absence of over acute toxicity.

Osakabe *et al.*, (2004) studied the effect of proanthocyanidins derived from cacao on diabetes-induced cataract formation in rats. They have tested whether dietary supplementation with CLP prevents cataract formation in rats with diabetes- induced by streptozotocin (STZ), using histological, histochemical, and biochemical analysis. Starting at 7 days after streptozotocin challenge, the animals were fed either a normal diet or a diet containing 0.5% w/w CLP over 10 weeks. Antioxidant status as assessed by measuring lipid peroxide production in plasma in response to azo compounds was lower in the STZ-rats fed control diet than in animals fed CLP. Opacity was first detected in the lenses of the control dietary group 5 weeks after STZ injection and cataracts had developed in the majority of these animals by 10 weeks. These changes were rarely

seen in the STZ/CLP diet group. Their findings suggest that CLP inhibits diabetes-induced cataract formation possibly by virtue of its antoxidative activity.

Maiti *et al.*, (2004) reported that the Indian traditional system of medicine and herbal remedies are prescribed for the treatment of diseases including diabetes mellitus. In recent years, plants are being effectively tried in a variety of pathophysiological states. *Tamarindus indica* Linn. is one of them. In the study, aqueous extract of seed of *Tamarindus indica* Linn. was found to have potent antidiabetogenic activity that reduces blood sugar level in streptozotocin (STZ)-induced diabetic male rat. The results were found a significant diminution in body weight of the animals in diabetic group in comparison to control. After aqueous seed extract of *Tamarindus indica* supplementation for 7 days the body weight was recovered significantly butnot to the control level. After 14 days of this supplementation, the body weight of all the

animals was insignificantly different from control level. The study suggests that *Tamarindus indica* may have beneficial effects in type-I diabetes mellitus that holds the hope of new generation of antidiabetogenic drugs.

Suryanarayana *et al.*, (2003) studied the effect of curucmin on galactose -induced cataractogenesis in rats. Cataract progression due to galactose feeding was monitored by slit lamp microscope and classified into 4 stages. At the end of the experiment biochemical parameters such as lipid peroxidation, aldose reductase (AR), sorbitol dehydrogenase (SDH), reduced glutathione, protein content and protein carbonyls were measured in the lens and crystallin in profile was analyzed by size exclusion chromatography (HPLC). Slit lamp microscope observations indicated that curcumin at 0.002% delayed the onset and maturation of cataract.Biochemical analysis showed that curcumin at the 0.002% level appeared to exert antioxidant and antiglycating effects,

as it inhibited lipid peroxidation, AGE-fluorescence, and protein aggregation. These results suggest that curcumin is effective against galactose-induced cataract only at a very low amount (0.002%) in the diet. On the other hand, a dose above 0.01% level seems to be beneficial under hyperglycemic conditions, at least with the model of galactose-cataract.

Lee *et al.*, (1999) reported the contributions of polyol pathway to oxidative stress in diabetic cataract. Using transgenic mice that over expresses aldose reductase (AR) in their lenses, they found that the flux of glucose through the polyol pathway is the major cause of hyperglycemic oxidative stress in this tissue. The substantial decrease in the level of reduced glutatione (GSH) with concomitant rise in the level of lipid peroxidation product malondialdehyde (MDA) in the lens of transgemic mice, but not in the nontrangenic mice, suggests that glucose auto oxidation and non enzymatic glycation do not

contributes to oxidative stress in diabetic lenses. AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its co factor NADPH, which is also required for the re-generation of GSH. Sorbitol dehydrogenase, the second enzyme in the polyol pathway that converts sorbitol to fructose, also contributes to oxidative stress, most likely because depletion of its co-factor NAD+ leads to more glucose being channeled through the polyol pathway. Despite a more than 100% increase of MDA, oxidative stress plays only a minor role in the development of cataract in the acute diabetic cataract model. However chronic oxidative stress generated by the polyol pathway is likely to be an important contributing factor in the slow – developing diabetic cataract as well as in the development of other diabetic complications.

Sugiyama *et al.*, (1999) reported that aldose reductase catalyzes the oxidation of naphthalene - 1, 2-

dihydrodiol for the formation of ortho-naphthoquione. The oxidation of naphthalene -1, 2-dihydrodiol (ND) to *O*-naphthoquinone (NQ) in the lens is believed to be responsible for the formation of cataracts in naphthalene-fed rats. Studies using either recombinant rat lenses (RLAR) or human muscle aldose reductase (HMAR) incubated *in vitro* with ND in the presence of NADP verified that aldose reductase is the dihydrodiol dehydrogenase that catalyses the oxidation of ND to NQ. Kinetic studies of V_{max}/ k_m indicated that RLAR catalyzes the NAD-dependent oxidation of ND with an optimal pH of 9.0. The corresponding activity of HMAR was lower than that of rat enzyme. The metabolite produced by the incubation of RLAR with ND in 20 mM phosphate buffer (pH 7.5) was isolated by C_{18} reversed-phase high performance liquid chromatography. The elution profile showed the formation of a new peak that was identical with a peak generated when NQ was incubated under same condition. The metabolite in both peaks was identified as 4-(2-

hydroxyethylsulfanyl)-1,2-dihydro-1,2-naphthalenedione (HNQ) by H^1 and C^{13} NMR analyses using homonuclear correlation spectroscopy, heteronuclear multiple quantum coherence, and heteronuclear shift correlations via multiple bond connectivities as well as infrared analysis. HNQ is readily autoxidised to 2, 3-dihydro-1-oxa-4-thia-9, 10-phenanthrenedione. The stoichiometry of 1:1 between the consumption and the formation of NADH for the formation of HNQ implies that rat lens aldose reductase catalyzes a $2e^-$ oxidation of ND to yield the corresponding ketol, which is autoxidized to NQ.

Lee *et al.*, (1998) reported the involvment of aldose reductase in naphthalene cataract. Lenses from nontransgenic mice and from transgenic lines CAR 222 and CAR 648, with different levels of AR were cultured in medium using naphthalene-1, 2 dihydrodiol with or without AR inhibitor AL 1576. The morphology and progression rate of ND–induced cataract in these lenses

were compared. Lenses from transgenic mice CAR 222 and CAR 648, but not their nontransgenic littermates, developed yellow pigment in the inner cortex when exposed to 50 μm ND, which was completely prevented by 0.2 mM AL 1576. The yellow Pigment developed faster and more intensely in the CAR 648 lens, which has a higher AR level than CAR 222. Under a high dose of 500 μm ND both transgenic and wild type mouse lenses developed ND-induced cataract, although the first sign of cataract was found in the outer cortex in transgenic lenses instead of the inner cortical region in wild type lenses. In addition, the cataract was more severe and developed at a faster rate in transgenic mouse lenses. AL 1576 showed only partial protection against the cataract induced by 500μm ND. The findings showed that the progression rate of ND –induced cataract correlated with the level of lens AR and ND, indicating that AR was the key, enzyme for the metabolism of ND in the process of naphthalene cataract development.

Kovaceva *et al.*, (1997) reported the difference in activities of antioxidant superoxide dismutase, glutathione peroxidase and prooxidant xanthine oxidoreductase /xanthine oxidase in the normal corneal epithelium of various mammalia. The enzyme activities of antioxidant superoxidase dismutase and glutatione peroxidaxe as well as proxident xanthine oxido reductase /xanthine oxidase were examined using biochemical methods. Results show that superoxide dismutase activity is high in rabbits and guinea pigs where as in pigs the activity is low and in cow it is nearly absent. In contrast, glutathione peroxidase activity is high in cows, pigs and rabbits, where as in guniea pig that activity is low. The findings for proxidant enzymes level reveal elevated xanthine oxidoredutase /xanthine oxidase activities in rabbits, lower activities in guinea pigs, very low activity in cows and no activity in pigs. In conclusion the results demonstrate inter species variations in activities of enzymes participating in

antioxidant/ pro-oxidant balance in the corneal epithelium. It is suggested that the levels of anti oxidant and pro-oxidant enzymes studies in the corneal epithelium might be associated with the diurnal or nocturnal activity of animals.

Gupta *et al.*, (1997b) reported that topical aspirin provides protection against galactose induced cataract. Effect of twice daily administration of aspirin eye drops on the onset and progression of cataract-induced by 30% galactose diet was studied. On the 30th day of galactose feeding, while all control group rats showed complete state IV opacity, those receiving aspirin eye drops showed only mild cataractous changes of stage I. *In vitro* studies showed that addition of aspirin to the medium significantly decreased dulcitol formation ($P<0.01$) and maintained glutathione levels ($P<0.02$). Intraocular penetration studies using isolated goat cornea should excellent penetration by salicylate indicating feasiblity of topical administration.

The results of the present study demonstrate that topical aspirin possesses significant anticataract activity in galactosemic cataract.

Vani and Rawal, (1996) studied the effect of riboflavin supplementation on glutathione and glutathtione redox cycle in selenite-induced cataractous lenses. The alterations in the level of proteins, reduced glutathione (GSH) and the activity of y-glutamylcysteine synthetase (y-GCS), glutathione reductase (GR) and glutathione peroxidase (GSH-px) have been studied in the control and riboflavin supplemented rats. The cataractous littermates supplemented with riboflavin showed increased activity of the enzymes and elevated levels of metabolites as compared to the cataractous, non-supplemented littermates. The results of study point towards the role of riboflavin in the prevention of cataract, induced by selenite.

Micelli-Ferrari *et al.*, (1996) evaluated the role of lipid peroxidation in the pathogenesis of myopic and senile cataract. The study was conducted on 34 lenses (nucleus and epinucleus) (9 clear lenses, 14 lenses with idiopathic senile cataract and 11 lenses affected by severe myopic cataract) and vitreous of 19 (7 non-myopic, 7 myopic, and 5 control) subjects. Glutathione and malondialdehyde was assayed. Cataractous lenses showed a decreased content of GSH and increased concentration of GSSH compared with clear lenses. A higher oxidative consumption of GSH was found in myopic cataracts compared with senile ones. Also, increased levels of MDA were observed both in cataractous lenses and in the vitreous of myopic patients compared with control and the senile ones. The observed alterations strongly suggest that retinal lipid peroxidation might play a key role in human cataractogenesis, especially in the myopic type.

Sato (1993) investigated the effect of aldose reductase, the major protein associated with naphthalene dihydrodiol dehydrogenase activity in rat lens. Aldose reductase was purified from whole rat lenses using a series of chromatographic steps that include gel filtration, affinity chromatography, and chromatofocusing. The dehydrogenase activity of the purifed enzyme was evaluated with 1, 2-dihydroxynaphthalene (naphthalene dihydrodiol) as substrate. The same dehydrogenase activity was also examined with the recoimbinent protein obtained from rat lenses aldose reductase clone. Both the reductase and dehydrogenase activites of purifed aldose reductase was inhibited by aldose reductase inhibitors However, inhibition of dehydrogenase activity was less than reductase activity. Aldose reductase displays dehydrogenase activity in addition to reductase activity. In rat lenses aldose reductase is a major protein associated with naphthalene dihydrodiol dehydrogenase

activity. This suggests that aldose reductase is linked to 1, 2-dihydroxynaphthalene formation in rat lens and the subsequent formation of cataracts in naphthalene-fed rats.

Tao *et al.*, (1991a) reported the effect of aldose reductase inhibitors on naphthalene cataract formation in rats. Brown Norway rats were administered naphthalene by gavage at a dose of 0.7 g/kg were fed normal rat chow containing aldose reductase inhibitors sorbinil, FK 366, A 11576 and 0.05% Tolrestat and Ponalrestat to inhibit sugar cataract formation the lens changes in these rats were monitored over a 90- day period by portable slit – lamp microscopy and histologic study. The compound A11576 showed a dose-dependent reduction in naphthalane-induced cataract formation, with no naphthalene-assosiated deposits seen in toluidine blue-stained lens sections. Sorbinil also reduced lens changes, whereas tolrestat ,ponalrestat ,and FK366 had no effect.

These results suggest that inhibition of naphthalene-induced cataract formation by structurally diverse aldose reductase inhibitors was not linked to the inhibition of aldose reductase.

Tao *et al.*, (1991b) compared the effect of two types of aldose reductase inhibitors on several biochemical parameters in naphthalene-induced cataract of the rat over a time span of 102 days of treatment. Feeding of naphthalene daily to brown Norway rats resulted in gradual, progressive development of zonular opacities. As compared to control animals, the values of soluble protein, soluble glutathione, glutathione peroxidase, glutathione reductase were decreased in rats fed either naphthalene or naphthalene +FK366, a carboxylic-acid-type aldose reductase inhibitor. In marked contrast, treatment with A11576, a hydantoin-type aldose reductase inhibitor, maintained the values of most parameters at levels that were similar to those of the

controls, and all lens remained clear. A decline of glutathione was noted in all naphthalene-fed rats, irrespective of whether these animals ha d been treated with aldose reductase inhibitor. The great decrease of glutathione with A11576 suggests that this inhibitor acts at some step in naphthalene metabolism following formation of naphthalene epoxide.

Datiles *et al.*, (1982) studied the effect of sorbinil, an aldose reductase inhibitor on cataract induced by galactose using a light microscopic study. Cataract formation in galactosemic rats was studied by ophthalmoscopy, slit-lamp biomicroscopy and by light microscopy using plastic embedding with methacrylate. Untreated rats developed nuclear cataract by 14 days and mature cataracts by 21 days However, rats treated with the aldose reductase inhibitor sorbinil did not develop any cataractous change for upto 8 months of 50% galactose feeding and could not be distingushied from

normal controls. This strongly suggests that aldose reductase is the common factor involved in the formation of sugar cataracts.

Chylack *et al.*, (1969) carried out a biochemical evaluation of a cataract induced in a high glucose medium. Rabbit lenses were incubated in low and high glucose media in an attempt to evaluate the role of the sorbitol pathway in the production of sugar cataract. The aldose reductase inhibitor 3, 3- tetramethylene glutaric acid (TMG), was employed to block sorbitol formation. Exposing lenses is high glucosse lead to an initial linear increase in sorbitol Content lens water. During the first four days of incubation, lens swelling occurs in response to intracellur sorbitol accumulation. Swelling renders cell membranes more permeable to sodium and potassium. Lens sodium rises and lens potassium falls and net result is an increase in total cations. At a somewhat later stage, the inulin space of the lens increases as lens fibers rupture

and /or become more permeable to inulin. The addition of TMG to high glucose medium practically abolishes sorbitol accumulation; it depresses lens swelling, preserves normal cation balance and maintained lens clarity and transparency for eight days. This suggests that all of the aforementioned changes are interrelated and also emphasizes the primary role played by aldose reductase in the intiation of the entire sequence of cataractous stage.

Heyningen and Pirie (1967) investigated the metabolism of naphthalene and its toxic effect on the eye. Rabbits were fed with 1g/kg of napthalene orally. Dissection of eye tissues revealed browning of the lens and eye humours, blue fluorescence of the eye humours and crystals in the retina and vitreous body. There was also a decrease in the humours. The metabolites of naphthalene finally are converted 1, 2 –naphthaquinone and hydrogen peroxide which accounts for the

disappearance of ascorbate, appearance of crystals of calcium oxalate in the eye and the brown colour of lens.

Sippel (1966) reported the changes in the water, protein, and glutathione contents of the lens in the course of galactose cataract development in rats. Four week old female rats fed a diet of ground chow diluted equally with galactose develop equatorial vacuoles in their lenses in 2 days and nuclear opacities in 12 to 14 days. By measurement of lens density, excessive hydration is detected by 12 h after the diet is started. The glutathione level is significantly decreased after another 12 h. The total protein content remains constant until nuclear cataract appear, and then decreases to half. At this time the absolute water content, which had increase nearly threefold, also decreases but to a lesser extent; the cataract is thus highly hydrated. By the time vacuolization is only slightly advanced (3 days), nine tenths of the original glutathione has disappeared. The remainder

persists through the subsequent stages of cataract development and probably resides in the proliferating epithelium and new fibers. The loss of glutathione is of unknown cause, may initially be a highly localized phenomenon, and is possibly related to previously reported changes in properties of lens proteins. Since osmotic vacuolization does not appear to be the immediate cause of nuclear opacification and subsequent membrane destruction, such protein changes may be of considerable importance.

OBJECTIVE AND PLAN OF WORK

Objective of the study

Cataract, an opacity of the lens, is the leading cause of blindness worldwide. It is estimated that there are about 12 million blind people due to cataract in India alone. Although various risk factors have been identified in the pathogenesis of senile cataract, oxidative damage to the constituents of the eye lens is considered to be one of the major mechanisms (Kanski, 2003) Cataractogenic degeneration of the lens causes oxidation of the cellular and membrane constituents. The toxic effects of reactive oxygen species (ROS) or free radicals are neutralized in the lens by both enzymatic and non-enzymatic antioxidants. In recent years, the role of alternative therapeutic approaches has become very popular to delay or counter complications such as cataract. Several studies suggest the use of medicinal plants having antioxidant potential is beneficial against cataract.

Tamarindus indica Linn belonging to the family Fabaceae is a large sized tree widely distributed in India. The leaves are reported to possess flavonoids, saponins and tannins. The leaves of this species are used in Indian traditional medicine for the treatment of ophthalmia and other eye infections, applied to reduce inflammatory swellings, tumours, etc (Krithikar and Basu, 1987).

The main objective of the present study is to screen the hydromethanolic extract of *Tamarindus indica* Linn. for its *in vitro* anticataract activity against glucose-induced cataractogenesis using goat lenses.

Plan of work

The work involved the following steps,

- Collection and authentification of the leaves of *Tamarindus indica* Linn.
- Preparation of hydromethanolic extract
- Phytochemical screening
- Induction of cataract by incubation of goat lenses with glucose (55 mM) for 72 h with and without the plant extract
- Estimation of tissue protein, malondialdehyde and lipid hydroperoxides in lens homogenate
- Esimation of enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, peroxidase and glutathione reductase and the non-enzymatic antioxidant, reduced glutathione in lens homogenate
- Statistical analysis.

MATERIALS AND METHODS

Plant material

The plant material consists of dried powdered leaves of *Tamarindus indica* Linn. belonging to the family Fabaceae.

Plant collection and authentication

The leaves of *Tamarindus indica* Linn were collected from Coimbatore district in Tamil Nadu, India during the month of June 2008. The plant was identified and authenticated by Mr. G.V.S. Murthy, Joint Diretctor, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SC/5/23/08-09/Tech-659.

Preparation of extraction

The fresh leaves of *Tamarindus indica* Linn are collected, dried in shade under room temperature, powdered mechanically and sieved through No. 20 mesh

sieve. The finely powdered leaves were kept in an airtight container until the time of use. The extraction was carried out by continuous hot percolation method using Soxhlet apparatus. The solvent used was a mixture of methanol: water in the ratio of 7:3. About 100 g of powder was extracted with 600 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50 °C.

Drugs and chemicals

Glucose and vitamin E were obtained from SD fine chemicals, Mumbai. Liquid paraffin was obtained from Fisher Chemicals Ltd., Chennai. Thiobarbituric acid, Trichloro aceticacid, Butylated hydroxyl toluene, oxidized glutathione, epinephrine and 5 5'Dithiobis-2 nitrobenzoic acid were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. 2 – 2' dipyridyl and *O*-dianisidine were obtained from Himedia Laboratories Ltd., Mumbai. Goat

lenses were obtained from the slaughterhouse Coimbatore. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Phytochemical screening

Chemical tests were carried out for the extract of *Tamarindus indica* Linn for the presence of phytochemical constituents (Trease and Evans, 2002).

Test for tannins and phenolics

To the solution of the extract, a few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of an emulsion.

Test for flavanoids

a) To a portion of the extract, concentrated H_2SO_4 was added. A yellow colouration observed indicates the presence of flavanoids. The yellow coloration disappeared on standing.
b) Few drops of 1% $AlCl_3$ solution was added to a portion of extract. A yellow coloration indicates the presence of flavonoids.
c) A portion of the extract was heated with 10 ml of ethylacetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1ml

of dilute ammonia solution. A yellow coloration indicates a positive test for flavonoids.

Test for terpenoids

About 5 ml of the extract was treated with 2 ml of chloroform and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface formed shows the presence of terpenoids.

Test for alkaloids

A small portion of the extract was stirred with few drops of dil HCl and filtered.

a) To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.
b) To the filtrate, Dragendorff's reagent (potassium bismuth iodide

solution) was added and an orange brown precipitate indicates the presence of alkaloids.

In vitro experimental model of cataract

In vitro experimental model of cataract was induced in goat lenses using glucose at a concentration of 55 mM, incubated for 72 h at room temperature. At high concentrations, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This leads to cataractogenisis.

Experimental protocol

A total of 30 goat lenses were used and divided into the following 5 groups (n = 6 in each group),

Group I: Artificial aqueous humor alone (solvent control)

Group II: Glucose 55 mM alone (Negative control)

Group III: Plant extract (100 µg/ml) + glucose 55 mM

Group IV: Plant extract (200 µg/ml) + glucose 55 mM

Group V: Vitamin E (100 µg/ml) + glucose 55 mM (Standard drug)

In vitro lens culture

Fresh goat eyeballs were collected from slaughterhouse, immediately after slaughter and transported to the laboratory. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaH(PO_4)_2$ 0.5 mM, $CaCl_2$ 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination (Langade, 2006).

Preparation of lens homogenate

After 72 h of incubation, homogenate of lenses (10% w/v) was prepared in Tris buffer (0.23 mM, pH 7.8) containing $0.25x10^{-3}$ M EDTA. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of total protein (TP), determination of the end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), and glutathione peroxidase (GPx), and the non-enzymatic antioxidant reduced glutathione (GSH).

Estimation of total protein (TP)

The amount of total protein present in the tissue homogenate was estimated by the method of Lowry *et al.*, 1951. To 0.1 ml of tissue homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very

rapidly and mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV–visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue (Lowry *et al.*, 1951).

Estimation of malondialdehyde (MAD)

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of tissue homogenate (Tris HCl buffer, pH 7.4) was treated with 2 ml (1:1:1 ratio) of TBA – TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 g at room temperature for 10 min. The absorbance of the

clear supernatant was measured against a reference blank at 535 nm. The values are expressed as nmoles of MDA /min/mg lens protein.

Estimation of lipid hydroperoxides (LH)

About 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250 mM sulphuric acid) and incubated for 30 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/mg lens protein (Nieshus and Samuelsson, 1986).

Determination of enzymatic antioxidants

Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence

of the homogenate at 480 nm. The reaction mixture contained 150 µl of lens homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate (Kakkar *et al.*, 1984).

Estimation of catalase (CAT)

The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1ml of tissue homogenate and was incubated at 37^0C for 15 min and the reaction was started with the addition of 0.1ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. Activity was expressed as µmoles/mg tissue protein (Abei, 1984).

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml (10 mg/ml) of bovine serum albumin (BSA). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 min against a blank. Glutathione reductase activity was expressed as nmoles NADPH oxidized /min/mg lens protein at 30^0C (Racker, 1955).

Estimation of peroxidase (Px)

Peroxidase activity was measured spectrophotometrically by following the change in

absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H_2O_2 and enzyme. Reaction mixture contained 0.2ml of 15 mM *O*-dianisidine, 0.1ml of tissue homogenate and 2.5 ml of 0.1M potassium phosphate buffer pH 5.0 and were incubated at 37^0C for 15 min and the reaction was started with the addition of 0.2 ml of hydrogen peroxide and the absorbance at 460 nm was followed against a blank, spectrophotometrically for about 3-5 min at 37^0C. Unit of enzyme activity defined as µmoles of *O*-dianisidine/min at 37^0C (Lobarzewski and Ginalska, 1995).

Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by the procedure of Paglia and Valentine, 1967. The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of supernatant incubated at 37^0C

for 10 min. The reaction was arrested by the addition of 10 % TCA and the absorbance was measured at 340 nm. Activity was expressed as nmoles/min/mg lens protein.

Determination of non enzymatic antioxidant

Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate, 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412nm. Activity was expressed as nmoles/min/mg lens protein (Ellman, 1959).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of six lenses in each group. P values < 0.05 were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening of the powdered leaves of *Tamarindus indica* showed the presence of phenolics, tannins, saponins, flavonoids and alkaloids.

Table 1. Phytochemical screening

Phytochemicals	***Tamarindus indica***
Alkaloids	+
Flavonoids C-glycosides	+
Saponins	+
Tannins and phenolics	+
Terpenoids	+

Effect of the extract of *Tamarindus indica* leaves on lens protein and lipid peroxidation in experimental groups

There was a significant (P<0.01) decrease in the level of total protein and an increase in the level of malondialdehyde and lipid hydroperoxides in glucose-induced cataractous lenses when compared to normal control. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 µg/ml) and Vitamin E (100 µg/ml) simultaneously with glucose for 72 h caused a significant (P<0.01) increase in the total protein and a decrease in the level of malondialdehyde and lipid hydroperoxides (Table 2).

Table 2. Effect of *Tamarindus indica* hydromethanolic leaf extract on lens protein, MDA and LH in control and experimental groups

GROUP	DOSE	PROTEIN	MDA	LH
Normal control	_	103.6±8.89	0.68±0.024	4.13±0.37
Glucose control	55 mM	36.64±1.26[a]	1.98±0.017[a]	10.98±0.60[a]
Plant extract	100 µg/ml	74.38±2.12[b]	0.84±0.014[b]	5.16±0.37[b]
Plant extract	200 µg/ml	76.22±3.12[b]	0.78±0.032[b]	4.88±0.73[b]
Vitamin-E	100 µg/ml	84.24±3.62[b]	0.72±0.08[b]	4.54±0.23[b]

Values are mean ± SEM; n=6 in each

[a]P <0.01 when compared to normal control;

[b]P<0.01 when compared to glucose control (one way ANOVA followed by Dunnett's test).

Protein = mmoles/min/mg , MDA = nmoles/min/mg protein, LH = nmoles/min/mg protein.

Effect of *Tamarindus indica* hydromethanolic leaf extract *on* lens enzymatic and non enzymatic antioxidants in experimental groups

Incubation with glucose 55 mM for 72 h produced a significant (P<0.01) decrease in the enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens homogenate when compared to normal control. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 µg/ml) and Vitamin E simultaneously with glucose significantly (P<0.01) restored the levels of both enzymatic and non enzymatic antioxidant enzymes which is almost similar to the control group (Table 3).

Table 3. Effect of *Tamarindus indica hydromethanoli* leaf extract on lens enzymatic and non enzymatic antioxidants in control and experimental groups

Group	Dose	Catalase	GPx	SOD	GSSH	Peroxidase	GSH
Normal control	_	1.73±0.17	2.88±0.29	4.53±0.23	1.99±0.09	2.47±0.32	2.99±0.32
Glucose control	55 mM	0.49±0.04[a]	1.12±0.11 [a]	1.23±0.37[a]	0.08±0.016[a]	0.86±0.19[a]	1.11±0.20[a]
Plant extract	100 µg/ml	1.12±0.09[b]	2.26±0.21 [b]	3.59±0.73[b]	0.94±0.26[b]	1.89±0.04[b]	2.17±0.23[b]
Plant extract	200 µg/ml	1.38±0.09[b]	2.43±0.24 [b]	3.84±6.37[b]	1.13±0.08[b]	1.96±0.13[b]	2.29±0.12[b]
Vitamine-E	100 µg/ml	1.42±0.07[b]	2.54±0.25[b]	3.92±0.60[b]	1.24±0.08[b]	2.10±0.21[b]	2.37±0.21[b]

Values are mean ± SEM; n = 6 in each group.

[a]P<0.01 when compared to normal control;

[b]P <0.01 when compared to glucose control (One way ANOVA followed by Dunnett's test).

CAT = µmoles/min/mg protein GPx = nmoles/min/mg protein, GSH = nmoles/min/mg protein,

SOD = nmoles/min/mg protein, GSSH = nmoles /min/mg protein and Peroxidase = nmoles/min/mg protein.

DISCUSSION AND CONCLUSION

The present thesis entitled "*In vitro* anticataract activity of *Tamarindus indica* L. against glucose-induced cataractogenesis" deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Tamarindus indica* L. belonging to the family Fabaceae, which is traditionally used by the local people and tribals in India for the treatment of inflammatory swelling, tumors, ringworm; useful in disease of blood, small pox, ophthalmia and other eye diseases, earache, snake-bike (Krithikar and Basu, 1981).

Cataract is one of the leading causes of visual disability often leading to blindness. It is an age-related phenomenon over and above, oxidative stress also plays an important role. The situation can be remedied surgically by extirpation of the cataractous lens. The limitations of cataract surgery have stimulated experimental cataract research in laboratory animals and epidemiological studies to determine the incidence,

prevalence and risk factors for the development of cataract so as to focus on the preventive aspects of cataract (Gupta *et al.*, 1997a)

Cataract was induced *in vitro* with glucose at a concentration of 55 mM in aqueous humor media and incubated for 72 h at room temperature (Langade 2006). After incubation the lens homogenate was used for the estimation of total protein (TP) content, determination of end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), glutathione peroxidase (GPx), and non-enzymatic antioxidant reduced glutathione (GSH).

Lipid peroxidation is an autocatalytic process, which is a common cause of cell deth (Bandhyopadhyay *et al.*, 1999). In order to elucidate the protective mechanism of the leaves of *Tamarindus indica*, glucose-induced goat

lens was examined for lipidperoxide levels. Decomposition of lipid peroxides initiate the chain reactions that produce reactive carbonyl compounds. The by-products of lipid peroxidation are the toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross linking ability. Lens MDA may be the result of lipid peroxidation of the lens cell membrane or may represent the consequence of its migration from the readily peroxidizable retina or from the central compartment. In our studies, glucose-induced goat lenses showed an increase in malondialdehyde and lipid hydroperoxide levels in lens. Incubation at different concentrations (100 & 200 µg/ml) of extract of *Tamarindus indica*, simultaneously with glucose (55 mM) for 72h caused a significant ($P<0.01$) decrease in the lens malondialdehyde and lipid hydroperoxides and an increase in total protein level. This effect was almost similar to the vitamin E treated group.

Several varieties of toxic species of oxygen are formed in the lens milieu, including superoxide anion, hydrogen peroxide, hydroxyl radical and lipid hydroperoxides. The enzyme catalyses the reduction of oxygen (during reperfusion phase), leading to the formation of superoxide and H_2O_2 as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations (Nijveldt ***et al.,*** 2001). Thus the determination of the lens ***in vitro*** antioxidant enzymes like SOD, CAT, GPx, GSSH, peroxidase (Px) and non enzymatic antioxidant enzyme, GSH were carried out.

Catalase is present in almost all the mammalian cells localized in the peroxisomes. It catalyses the decomposition of H_2O_2 to water and oxygen and thus proctects the cell from oxidative damage by H_2O_2 and hydroxyl radical. The dichromate/acetic acid reagent can be thought of as a 'stop bath for catalase activity. As soon as the enzyme hits the acetic acid, its activity is

destroyed, any H_2O_2 which is not split by the catalase will react with the dichromate to give a blue precipitate which is then decomposed on heating to give a green solution. *Tamarindus indica* extract significantly increased ($P<0.01$) the catalase level in glucose-induced cataractous lenses.

The first enzyme involved in the antioxidant defence is superoxide dismutase. It is metalloprotein found in both prokaryotic and eukaryotic cells. The oxygen radicals, generated by intraction of Fe^{2+} and H_2O_2 are the species responsible for the oxidation of epinephrine at pH 10.2 and was strongly inhibited by superoxide dismutase (Misra *et al.*, 1972).

GPx has a major role in degrading the levels of H_2O_2 in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration (Rotruk *et al.*, 1973). The

enzymatic antioxidant levels in glucose-induced cataract groups were decreased when compared to normal control group. The leaf extract of *Tamarindus indica* significantly (P<0.01) increased the level of antioxidant enzymes. which is almost similar to the vitamin E treated group.

GSH is an intracelluar reductant which plays major role in catalysis, metabolism and transport. It protects cell against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects (Gupta *et al.*, 1997a). Extract of *Tamarindus indica* significantly (P<0.01) increased the level of GSH when compared to glucose-induced cataractous lenses.

To conclude, the study suggested that the leaf extract of *Tamarindus indica* possess anticataract and antioxidant activities, which might be helpful in preventing

or slowing the progress of cataract. Further investigations on the isolation and identification of active components in the leaves may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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DEDICATED WITH LOVE TO MY MOST BELOVED PARENTS, BROTHERS & SISTERS & THE ALMIGHTY